Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Biosafety and Health xxx (2020) xxx

Contents lists available at ScienceDirect

Biosafety and Health

journal homepage: www.elsevier.com/locate/bsheal

"Genetic tuning" of avian influenza virus host adaptation from birds to humans

Yasuha Arai, Yohei Watanabe *

Department of Infectious Diseases, Graduate School of Medical Sciences, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan

Emerging viral infections are a growing threat to human health and often have originated from zoonotic diseases [1]. Viruses infecting animals can cross the interspecies barrier and infect humans by the evolution of genetic variants with more fit replication in mammals, which has been defined as "genetic tuning" [2].

All the influenza A virus subtypes have been isolated from wild birds. Influenza A viruses cross the avian-human species barrier by acquiring genetic mutations or reassortments that enable them to adapt to humans [3]. Several avian influenza viruses (AIVs) (e.g., H5N1, H7N7, H9N2, H7N9, H5N6 and H7N4) can infect humans and cause respiratory diseases with a range of severity [4]: these have been geographically centered in Asia and the Middle East. Mutations for adaptation to infect mammals have been mainly identified in the viral hemagglutinin (HA) surface antigen and in the viral polymerase PB2 subunit [3].

The best-characterized human adaptation mutation is the PB2-E627K substitution. Historically, past pandemic influenza viruses (i.e., Spanish influenza virus-1918, Asian influenza virus-1957, and Hong Kong influenza virus-1968) have acquired this mutation [3]. The PB2-E627K mutation enables an AIV polymerase to be catalytically more active in mammalian cells at lower temperature, allowing enhanced AIV replication in mice and in human upper respiratory tract. Although the mechanism underlying mammalian adaptation by E627K has not been determined, several theories have been proposed, including a structural alteration to regulate polymerase activity and usage of host factors like ANP32A [5]. Other mammal adaptation mutations have been identified in the PB2 C-terminal region (e.g., K526R, Q591K, E627V and D701N) [3,6–8].

The viral HA binds sialylglycan, which is the host cell membrane receptor for influenza viruses: HA recognizes the specific linkage between the terminal sialic acid and penultimate galactose [3]. AIVs need to change their HA binding preference from the avian-type α2,3 linkage to the human-type α2,6 linkage to efficiently infect humans. Other viral genes also can affect the host range of influenza viruses. An example is neuraminidase (NA), another viral surface antigen that functions opposite to HA and releases the virus from its sialylglycan receptor. Thus, a functional rebalancing between HA binding affinity and NA cleavage activity, which is sometimes linked with decreased NA activity, is critical for an AIV to adapt to infect mammals [9].

Most past studies of AIV adaptation dynamics to infect mammals have been in cell cultures and animal models [10–12]. However, there has been little information about the "genetic tuning" of AIVs in patients, because the dynamics of host adaptation at the bird-human interface by genetic mutations is so fluid in nature. Many questions must be answered about AIV adaptation to infect humans. For example, do more fit virus variants already exist in birds in the field as minor variants before they are transmitted to humans? How rapidly does a more fit virus variant emerge and become dominant in a new infected host? Is there any correlation between the emergence of a more fit variant in a clinical patient and the severity of the disease and/or the outcome in that patient?

Although the dynamics of virus adaptation to infect a different host species has been difficult to analyze, deep sequencing may offer a useful approach to address this problem. In a breakthrough achievement, Liu et al. [13] have directly deep sequenced the original virus-positive samples from 39 H7N9 influenza patients in China and from 38 poultry/environment samples from the patient-contacting live bird markets, and traced the dynamics of the nucleotide substitutions in their H7N9 PB2 sequences containing residue 627, which is a key amino acid residue for AIV adaptation to humans (See Fig. 1.).

To elucidate H7N9 virus "genetic tuning", Liu et al. used two indices: (1) the ratio of PB2-627K to PB2-627E (i.e., the sequence depth of 627K to 627E, designated the K/E ratio) and (2) the sequence depth of K and E in PB2-627 whole sequence depths (designated the K ratio and E ratio, respectively). The overall data on viral adaptation showed distinct modes of evolution for the PB2-E627K substitution between viruses from poultry and from humans. Patient samples had diverse K/E ratios ranging from 1,510:1 to 1:2,000 with divergent PB2 dominance (K ratio: 0 to 98.29%), indicating drastic adaptation dynamics within each infected individual. In contrast, a substantial fraction (31.6%) of surrounding poultry/environment samples had no PB2-627K even under high sequencing depths (> 1,000), with constant 627E dominance in all samples (E ratio: > 99%). The adaptation dynamics in infected patients was confirmed by the gradual replacement of 627E by 627K in longitudinally collected specimens from one H7N9 influenza patient. These data suggested that H7N9 host

* Corresponding author: Department of Infectious Diseases, Graduate School of Medical Sciences, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan.
E-mail address: nabe@koto.kpu-m.ac.jp (Yohei Watanabe).

http://dx.doi.org/10.1016/j.bsheal.2020.11.006

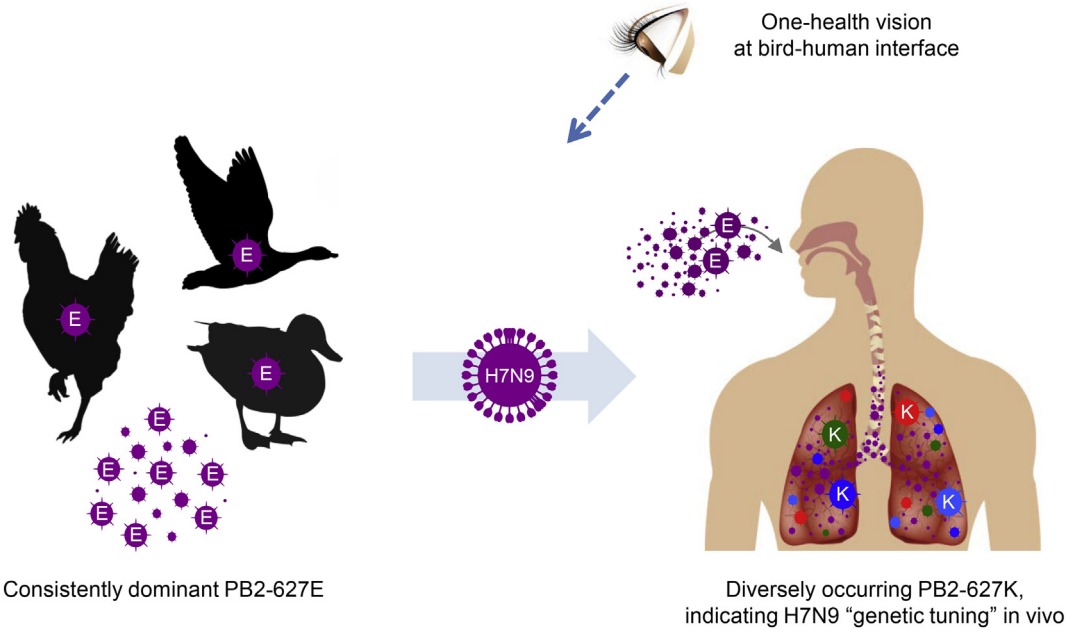

Fig. 1. Deep sequencing of H7N9 influenza viruses from patients and from their surrounding poultry/environment allowed analysis of the dynamics of H7N9 genetic mutations at the bird-human interface for host adaptation, providing the first in-depth data on H7N9 "genetic tuning" in clinical patients. This was a one-health approach for analyzing a zoonotic virus in the field and should be applicable to a wide range of emerging viruses and enable mitigation of public health risk.

adaptation to a more fit phenotype to infect a human host occurred in H7N9-infected patients. There was also more rapid host adaptation of the PB2-E627K mutation in fatal infection cases than in cases where the patient survived, indicating a correlation between H7N9 virus adaptation in patients and disease severity and outcome. These data on zoonotic virus "genetic tuning" during human infection are important for our understanding of AIV interspecies adaptation in the one-health concept.

Liu et al. [13] also identified some interesting dynamics of H7N9 adaptation in infected patients. First, PB2-E627K substitution became dominant in a substantial fraction (72.22%) of patients, but not in all patients. Therefore, what factor(s) caused different E627K substitution dynamics in different patients? AIV strains should carry nucleotide variations in their genomes, which may affect the temporal order or pattern of other nucleotide mutations during viral adaptation to infect humans. However, variance in the E627K substitution may reflect patient age, disease history, immune condition, and/or the time from the initial virus exposure to the specimen sampling. Second, the data also showed distinct substitution dynamics of the PB2-D701N mutation compared to the PB2-E627K mutation: no dominant 701 N was detected in all specimens. This indicated a less essential role for D701N in determining virus fitness during H7N9 adaptation to infect humans or that E627K emergence may have suppressed the subsequent emergence of other adaptive mutations, producing a sequential order in the mutations in patients. Future studies with animal infection models may answer these questions.

To make further progress in elucidating AIV adaptation to infect humans, it will be crucial to address some remaining questions. Ferret passage studies [11, 12] have shown that H5N1 AI variants have acquired a characteristic set of phenotypic changes by a series of HA mutations to be airborne transmissible. The H5N1 variants sequentially changed their receptor binding preference, pH threshold for membrane fusion, and HA stability to balance AIV adaptation for intra-host infection and inter-host transmission, which are distinct properties as reported previously [10]. Therefore, the rate at which such adaptive HA mutations occur, serially or simultaneously, in AIV-infected patients is one of the prime questions that remains to be elucidated.

Another question is the role of different compensating adaptive mutations. For example, past studies have suggested distinct roles for the PB2-D701N and PB2-K526R mutations when combined with the PB2-E627K mutation. The PB2-D701N and PB2-E627K mutations have not been detected together in an AIV in the field with a few exceptions, implying little synergism between these two mutations [7]. In contrast, the K526R/E627K (V) double mutation has been detected in AIVs in the field, with strong synergistic effects in H7N9 and H9N2 adaptation to infect mammals [6,8]. Thus far, there has been no in-depth data on the dynamics of multiple adaptive mutations during viral transmission between hosts in different species, which would be useful for understanding the compensatory roles of mutations in AIV adaptation. The deep sequencing approach of Liu et al. has the potential to answer these key questions. Indeed, Liu et al. identified co-emergence of the D701E and E627V substitutions in the same alleles in several H7N9 influenza patients, which implied a compensatory role or interdependency of such substitutions during H7N9 virus adaptation to infect humans. Additional studies are needed to elucidate the synergistic effect of D701E and E627V on virus properties (e.g., pathogenicity and transmissibility).

Deep sequencing does have some limitations. The fragmentation of PCR amplicons for deep sequencing complicates the analysis of multiple mutations that are at a substantial distance from each other in the same allele (i.e., PB2–526 and −627). Therefore, deep sequencing is regularly combined with multifaceted approaches (e.g., classical virological methods and Sanger sequencing). However, the study by Liu et al. [13] has opened the way toward a better understanding of the evolution of interspecies adaptation by zoonotic viruses. Deep sequence analysis can provide valuable data on "genetic tuning" in adaptation for interspecies infections of other zoonotic viruses, including SARS-CoV-2 [14] and the novel swine influenza virus reported in China [15]. A better understating of the adaptation dynamics that involves the interdependence between virus and host species may be useful for mitigation of future pandemic public health risks in a one-health approach.

Acknowledgements

The authors' research is supported by JST-CREST, Japan (Grant Number JPMJCR15F4) and the JST-MIRAI Program, Japan (Grant Number JPMJMI19D4), partly by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and

Technology (MEXT) of Japan (Grant Numbers 18K15171, 19H04841 and 20K21723, 20KK0224), and partly by grants from the Takeda Science Foundation, the Ichiro Kanehara Foundation, SENSHIN Medical Research Foundation, and a Sasakawa Scientific Research Grant from the Japan Science Society.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

[1] K.E. Jones, N.G. Patel, M.A. Levy, A. Storeygard, D. Balk, J.L. Gittleman, P. Daszak, Global trends in emerging infectious diseases, Nature 451 (2008) 990–993, https://doi.org/10.1038/nature06536.

[2] W. Zhu, Y. Shu, Genetic tuning of avian influenza A (H7N9) virus promotes viral fitness within different species, Microbes Infect. 17 (2015) (2015) 118–122, https://doi.org/10.1016/j.micinf.2014.11.010.

[3] J.S. Long, B. Mistry, S.M. Haslam, W.S. Barclay, Host and viral determinants of influenza A virus species specificity, Nat. Rev. Microbiol. 17 (2019) 67–81, https://doi.org/10.1038/s41579-018-0115-z.

[4] WHO, Avian Influenza Weekly Update, https://www.who.int/westernpacific/emergencies/surveillance/avian-influenza, 2020 (accessed 23 September 2020).

[5] A.R. Camacho-Zarco, S. Kalayil, D. Maurin, N. Salvi, E. Delaforge, S. Milles, M.R. Jensen, D.J. Hart, S. Cusack, M. Blackledge, Molecular basis of host-adaptation interactions between influenza virus polymerase PB2 subunit and ANP32A, Nat. Commun. 11 (2020) 3656, https://doi.org/10.1038/s41467-020-17407-x.

[6] Y. Arai, N. Kawashita, M.S. Ibrahim, E.M. Elgendy, T. Daidoji, T. Ono, T. Takagi, T. Nakaya, K. Matsumoto, Y. Watanabe, PB2 mutations arising during H9N2 influenza evolution in the Middle East confer enhanced replication and growth in mammals, PLoS Pathog. 15 (2019), e1007919. https://doi.org/10.1371/journal.ppat.1007919.

[7] S. Yamada, M. Hatta, B.L. Staker, S. Watanabe, M. Imai, K. Shinya, Y. Sakai-Tagawa, M. Ito, M. Ozawa, T. Watanabe, S. Sakabe, C. Li, J.H. Kim, P.J. Myler, I. Phan, A. Raymond, E. Smith, R. Stacy, C.A. Nidom, S.M. Lank, R.W. Wiseman, B.N. Bimber, D.H. O'Connor, G. Neumann, L.J. Stewart, Y. Kawaoka, Biological and structural characterization of a host-adapting amino acid in influenza virus, PLoS Pathog. 6 (2010), e1001034. https://doi.org/10.1371/journal.ppat.1001034.

[8] W. Song, P. Wang, B.W. Mok, S.Y. Lau, X. Huang, W.L. Wu, M. Zheng, X. Wen, S. Yang, Y. Chen, L. Li, K.Y. Yuen, H. Chen, The K526R substitution in viral protein PB2 enhances the effects of E627K on influenza virus replication, Nat. Commun. 5 (2014) 5509, https://doi.org/10.1038/ncomms6509.

[9] Y. Arai, E.M. Elgendy, T. Daidoji, M.S. Ibrahim, T. Ono, N. Sriwilaijaroen, Y. Suzuki, T. Nakaya, K. Matsumoto, Y. Watanabe, H9N2 influenza virus infections in human cells require a balance between NA sialidase activity and HA receptor affinity, J. Virol. 94 (2020), e01210-20. https://doi.org/10.1128/JVI.01210-20.

[10] Y. Watanabe, Y. Arai, N. Kawashita, M.S. Ibrahim, E.M. Elgendy, T. Daidoji, J. Kajikawa, H. Hiramatsu, N. Sriwilaijaroen, T. Ono, T. Takagi, K. Takahashi, T. Shioda, K. Matsumoto, Y. Suzuki, T. Nakaya, Characterization of H5N1 influenza virus quasispecies with adaptive hemagglutinin mutations from single-virus infections of human airway cells, J Virol. 92 (2018), e02004-17. https://doi.org/10.1128/JVI.02004-17.

[11] M. Imai, T. Watanabe, M. Hatta, S.C. Das, M. Ozawa, K. Shinya, G. Zhong, A. Hanson, H. Katsura, S. Watanabe, C. Li, E. Kawakami, S. Yamada, M. Kiso, Y. Suzuki, E.A. Maher, G. Neumann, Y. Kawaoka, Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets, Nature 486 (2012) 420–428, https://doi.org/10.1038/nature10831.

[12] S. Herfst, E.J. Schrauwen, M. Linster, S. Chutinimitkul, E. de Wit, V.J. Munster, E.M. Sorrell, T.M. Bestebroer, D.F. Burke, D.J. Smith, G.F. Rimmelzwaan, A.D. Osterhaus, R.A. Fouchier, Airborne transmission of influenza A/H5N1 virus between ferrets, Science 336 (2012) 1534–1541, https://doi.org/10.1126/science.1213362.

[13] W.J. Liu, J. Li, R. Zou, J. Pan, T. Jin, L. Li, P. Liu, Y. Zhao, X. Yu, H. Wang, G. Liu, H. Jiang, Y. Bi, L. Liu, K.Y. Yuen, Y. Liu, G.F. Gao, Dynamic PB2-E627K substitution of influenza H7N9 virus indicates the in vivo genetic tuning and rapid host adaptation, Proc. Natl. Acad. Sci. USA 2020 (2020), https://doi.org/10.1073/pnas.2013267117.

[14] WHO, Coronavirus Disease (COVID-19) Pandemic, https://www.who.int/emergencies/diseases/novel-coronavirus-2019, 2020 (acessed 19 October 2020).

[15] H. Sun, Y. Xiao, J. Liu, D. Wang, F. Li, C. Wang, C. Li, J. Zhu, J. Song, H. Sun, Z. Jiang, L. Liu, X. Zhang, K. Wei, D. Hou, J. Pu, Y. Sun, Q. Tong, Y. Bi, K.C. Chang, S. Liu, G.F. Gao, J. Liu, Prevalent Eurasian avian-like H1N1 swine influenza virus with 2009 pandemic viral genes facilitating human infection, Proc. Natl. Acad. Sci. USA 117 (2020) 17204–17210, https://doi.org/10.1073/pnas.1921186117.